Effect of bromelain and papain gel on enamel deproteinisation before orthodontic bracket bonding

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Aim: To test the hypothesis that enamel surface deproteinisation with different concentrations of bromelain in association with 10% papain increases the shear bond strength (SBS) of brackets bonded with orthodontic composite and resin modified glass ionomer cement (RMGIC).

Materials and methods: Orthodontic brackets were attached according to the following protocols to 195 bovine incisors, which were acquired and divided into 13 groups: 1) Transbond XT (TXT) according to the manufacturer's recommendations; 2) Deproteinisation with 3% bromelain (BD) plus 10% papain and TXT; 3) 6% BD plus 10% Papain and TXT; 4) RMGIC, without enamel deproteinisation and without acid etching; 5) RMGIC, with 3% BD plus 10% papain and without acid etching; 6) RMGIC, with 6% BD plus 10% papain and without acid etching; 7) attachment using RMGIC following etching with polyacrylic acid; 8) 3% BD plus 10% papain, attachment using RMGIC and etching with polyacrylic acid; 9) 6% BD plus 10% papain, and attachment using RMGIC following etching with polyacrylic acid; 10) etching with 37% phosphoric acid and attachment using RMGIC; 11) 3% BD plus 10% papain, etching with 37% phosphoric acid and attachment using RMGIC; 12) 6% BD plus 10% papain, etching with 37% phosphoric acid and attachment using RMGIC; 13) deproteinisation with 2.5% sodium hypochlorite (NaOCl), etching with polyacrylic acid and RMGIC. After bonding, the brackets were removed by a universal mechanical testing machine, which recorded shear bond strength at failure. The material remaining on the tooth was assessed using the adhesive remnant index (ARI).

Results: Deproteinisation with 3% and 6% bromelain gel plus papain significantly increased the shear bond strength ($p < 0.05$), when acid etching was performed with phosphoric acid, followed by primer application and attachment using Transbond XT (Group 3) and when attached with RMGIC without etching. Deproteinisation with 6% bromelain gel plus papain significantly increased ($p < 0.05$) the ARI score only when attachment was performed using RMGIC, without etching (Group 6).

Conclusions: Deproteinisation with bromelain associated with papain in a gel increased the shear bond strength and is recommended before orthodontic bracket attachment.

(Aust Orthod J 2016; 32: 23–30)

Received for publication: December 2014
Accepted: February 2016

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Introduction

The removal of organic matter (deproteinisation) from the enamel surface facilitates acid etching[1] and makes orthodontic bracket bonding to teeth more effective.[2,3] Sodium hypochlorite and papain have been used for this purpose due to their proteolytic characteristics.[1,2,4]

Bromelain is a proteolytic enzyme of the cysteine proteinase group[5] extracted from *Ananas comosus*, commonly known as pineapple, which is a member of the Bromeliaceae family of tropical plants. Its properties include anti-inflammatory,[6] anti-edeomatous,[7] anticoagulant, antibiotic,[8-10] antimetastatic[11] effects and also assistance in wound debridement.[12,13]

Bromelain has been used in dentistry and particularly in minor oral surgery since the 1960s for its anti-inflammatory action.[14-16] More recently, this substance has been used as the active ingredient in dentifrices for stain removal, particularly when combined with papain, and has demonstrated significant tooth whitening effects.[17] Papain is extracted from *Carica papaya*, popularly known as the papaya fruit.[18] It is also a proteolytic enzyme of the cysteine protease group and possesses antibacterial, anti-inflammatory and debris-removal properties that justify its use in the maintenance of health.[18-21] The debris removal effect is promoted by papain's high enzymatic specificity that acts only on necrotic tissues.

Papain is presented in a dental gel form combined with chloramine which together is used in the chemo-mechanical caries removal of infected dentin without damage to healthy tissues.[19,22-25] Papain has also been used in isolation and in association with chlorhexidine as an irrigant in root canal treatment.[26] In orthodontics, papain has shown significant potential in enamel deproteinisation before orthodontic bracket bonding.[2,3]

The aim of the present study was to determine whether bromelain, in association with papain, was capable of removing enamel surface protein before orthodontic bracket bonding and therefore improving bond strength.

Materials and methods

One hundred and ninety-five bovine permanent incisors, extracted and stored in 10% formaldehyde solution, were acquired. After seven days of fixation, the teeth were cleaned and the adhering periodontal tissue was removed. Teeth with obvious cracks and fractures were excluded from the sample. Following cleaning, the incisors were embedded in acrylic moulds measuring 25 × 20 mm (Tigre, Joinville, Brazil) using self-polymerising resin and subsequently stored in water under refrigeration.

Initially, the prophylaxis of all teeth was performed with pumice and water for 15 seconds followed by washing and drying for an equal length of time. The teeth were randomly divided into 13 groups (N = 15) and underwent varying bonding procedures according to the following protocols (Table I):

Group 1: 37% phosphoric acid etching for 15 seconds, followed by primer application and bracket attachment using Transbond XT (3M Unitek, CA, USA).

Group 2: deproteinisation with 3% bromelain plus 10% papain in gel form (Macela Dourada, Jequié, Brazil), 37% phosphoric acid etching for 15 seconds, followed by primer application and bracket attachment using Transbond XT.

Group 3: deproteinisation with 6% bromelain plus 10% papain in gel form, 37% phosphoric acid etching for 15 seconds, followed by primer application and bracket attachment using Transbond XT.

Group 4: bracket attachment with resin modified glass ionomer cement (RMGIC) (3M Unitek Multicure Glass Ionomer, CA, USA), without acid etching.

Group 5: deproteinisation with 3% bromelain plus 10% papain in gel form, bracket attachment using RMGIC, without acid etching.

Group 6: deproteinisation with 6% bromelain plus 10% papain in gel, bracket attachment using RMGIC, without acid etching.

Group 7: attachment using RMGIC following polyacrylic acid etching.

Group 8: deproteinisation using 3% bromelain plus 10% papain in gel form, followed by etching by polyacrylic acid and bracket attachment using RMGIC.

Group 9: deproteinisation with 6% bromelain plus 10% papain in gel form, followed by polyacrylic acid etching and attachment using RMGIC.

Group 10: 37% phosphoric acid etch and attachment using RMGIC.

Group 11: deproteinisation with 3% bromelain plus 10% papain in gel form, followed by 37% phosphoric acid etching and attachment using RMGIC.

Group 12: deproteinisation with 6% bromelain plus 10% papain in gel form, followed by 37% phosphoric acid etching and attachment using RMGIC.

Group 13: deproteinisation with 2.5% sodium hypochlorite, etching with polyacrylic acid and attachment using RMGIC.

The polyacrylic acid, phosphoric acid, bromelain gel and sodium hypochlorite solutions were applied on the vestibular surface of the enamel with an applicator brush for a period of 15 seconds followed by washing and drying. The orthodontic brackets were fixed to the centre of the crown with Transbond TX or resin modified glass ionomer cement. The excess Transbond XT or RMGIC was removed with a probe (Duflex, Juiz de Fora, Minas Gerais), following which, each bracket was polymerised using an LED light appliance (850 Mw/cm^2). After bracket attachment, all samples were stored in artificial saliva at a temperature of 37° for 24 hours.

A custom-made stand stabilised the teeth during the debonding tests. Each tooth was subjected to a shear load in a Universal Testing Machine model DL-10.000 (Oswaldo Filizzola, São Paulo, Brazil) delivered by a knife-edged blade at a crosshead speed of 0.5 mm/min. The force was applied to the orthodontic bracket base and parallel to the tooth surface and the shear load was recorded at the point of failure. The force per unit area required to dislodge the bracket was calculated and recorded as the shear bond strength (SBS) in megapascals (MPa).

The enamel surfaces were examined using a stereomicroscope (Stemi 2000-C, Carl Zeiss, Göttingen, Germany) under 16× magnification to determine the amount of composite resin remaining and subsequently classified according to the Adhesive Remnant Index (ARI). The ARI scores ranged from 0 to 3, with 0 indicating no composite left on the enamel; 1, less than half of the composite left; 2, more than half of the composite left; and 3, all of the composite remaining on the tooth surface. The examiner was calibrated to check intra-examiner variability and 15 enamel surfaces were randomly selected and re-analysed after an interval of 15 days.

Statistical analysis

For descriptive analysis of the shear strength and ARI scores, the means and standard deviations were calculated. For inferential analysis of the shear strength, the homogeneity of variances was tested by using the Levene test and the normality of the residues by the analysis of variance (one-way ANOVA) by using the Kolmogorov-Smirnov test. After data heterogeneity of variances and/or asymmetrical distribution of the residues were confirmed, the Kruskal-Wallis test was applied and pair comparisons performed using the Mann-Whitney test. The Kruskal-Wallis and Mann-Whitney tests were also applied to determine the differences in ARI scores between the groups. The level of significance adopted was 5% (α = 0.05). The data were tabulated and analysed using SPSS

Table I. Experimental groups.

	Enamel deproteinisation			Acid etching of enamel			Bonding material	
Groups	D.3%B +10%P	D.6%B +10%P	DNaClO	37% Phosporic acid etching	Without acid etching	Poliacrylic acid etching	Transbond XT	RMGIC
1	——	——	——	Yes	——	——	Yes	——
2	Yes	——	——	Yes	——	——	Yes	——
3	——	Yes	——	Yes	——	——	Yes	——
4	——	——	——	——	Yes	——	——	Yes
5	Yes	——	——	——	Yes	——	——	Yes
6	——	Yes	——	——	Yes	——	——	Yes
7	——	——	——	——	——	Yes	——	Yes
8	Yes	——	——	——	——	Yes	——	Yes
9	——	Yes	——	——	——	Yes	——	Yes
10	——	——	——	Yes	——	——	——	Yes
11	Yes	——	——	Yes	——	——	——	Yes
12	——	Yes	——	Yes	——	——	——	Yes
13	——	——	Yes	——	——		——	Yes

D. 3%B+10%P: Deproteinisation with 3% Bromelain + 10% Papain in gel (Macela Dourada, Jequié, Brazil)

D. 6%B+10%P: Deproteinisation with 6% Bromelain + 10% Papain in gel (Macela Dourada, Jequié, Brazil)

DNaClO: Deproteinised with 2.5% sodium hypochlorite

Transbond XT: Primer application and fixation with Transbond XT (3M Unitek, CA, USA)

RMGIC: fixed with resin modified glass ionomer cement (3M Unitek Multicure Glass Ionomer, CA USA)

Statistics for Windows software (IBM SPSS. 21.0, 2012, NY, USA).

Results

The effect of bromelain plus papain with phosphoric acid etching plus primer application and bracket attachment using Transbond TX

The results demonstrated that enamel surface deproteinisation with bromelain gel at concentrations of 3% or 6% plus 10% papain significantly increased bracket shear bond strength (Figure 1A) in comparison with the phosphoric acid-etched control group (Group 1). However, no significant differences were observed in shear bond strength when treatment used 3% or 6% bromelain. In addition, no significantly different effect on ARI scores of deproteinisation with bromelain gel plus papain was observed (Figure 1B).

The effect of bromelain plus papain and attachment with RMGIC without etching

The results demonstrated that enamel surface deproteinisation with bromelain gel at concentrations of 3% or 6% plus 10% papain significantly increased shear bond strength and the ARI score (Figure 2A) in comparison with the corresponding control group (Group 4) (Figure 2B) when attachment was provided by RMGIC without etching. No significant differences were observed in the shear bond strength and ARI scores between the treatments that used 3% or 6% bromelain.

The effect of bromelain plus papain with polyacrylic acid etching and attachment with RMGIC

The results demonstrated that enamel surface deproteinisation with bromelain gel at concentrations

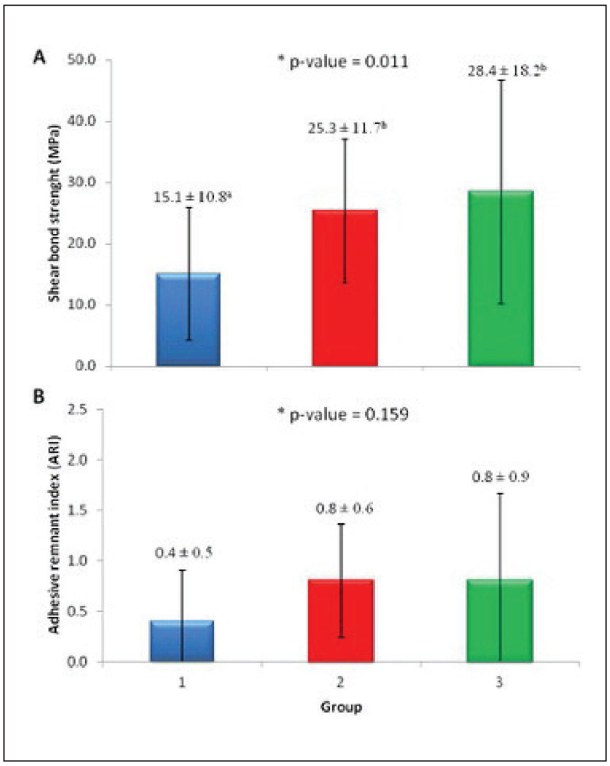

Figure 1. Comparison of shear bond strength (A) and ARI (B) between groups. Columns represent means, and error bars the standard deviations.

* Kruskal-Wallis Test; a,b Values with different superscript letters indicate significant differences between the groups (Mann-Whitney test); Group 1 (Control): phosphoric acid etching, followed by primer application and fixation with Transbond XT; Group 2: deproteinisation with 3% bromelain gel plus 10% papain plus phosphoric acid etching, followed by primer application and fixation with Transbond XT; Group 3: deproteinisation with 6% bromelain plus 10% papain plus phosphoric acid etching, followed by primer application and fixation with Transbond XT.

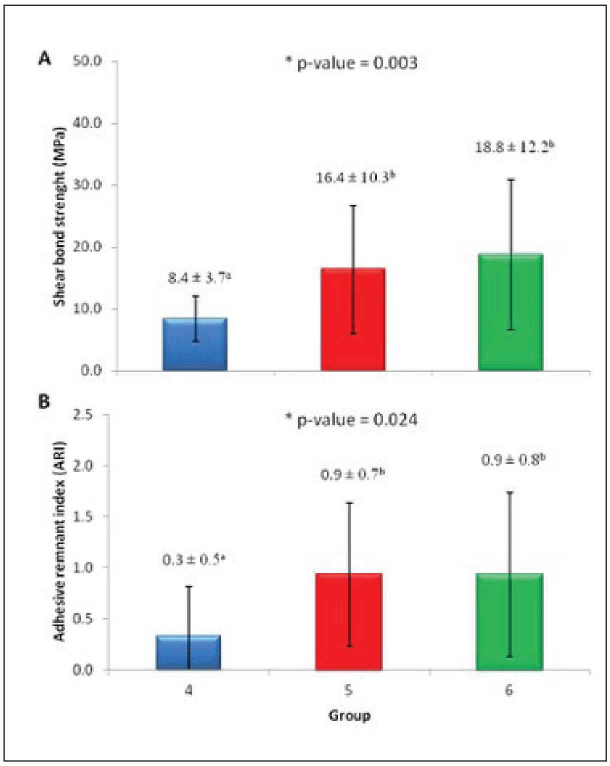

Figure 2. Comparison of shear bond strength (A) and ARI (B) between groups. Columns represent means, and error bars the standard deviations.

*Kruskal-Wallis Test; a,b Values with different superscript letters indicate significant differences between the groups (Mann-Whitney test); Group 4 (Control): fixation with RMGIC, without etching; Group 5: deproteinisation with 3% bromelain gel plus 10% papain and fixation with RMGIC, without etching; Group 6: deproteinisation with 6% bromelain gel plus 10% papain and fixation with RMGIC, without etching.

of 3% or 6% plus 10% papain did not increase shear bond strength (Figure 3A) and the ARI score (Figure 3B) when polyacrylic acid etching was performed and brackets were bonded using RMGIC.

The effect of bromelain plus papain with phosphoric acid etching and fixation with RMGIC

The results demonstrated that enamel surface deproteinisation with bromelain gel at a concentration of 3% or 6% plus 10% papain did not increase shear bond strength (Figure 4A) and the ARI score (Figure 4B) when phosphoric acid etching was performed and brackets were bonded with RMGIC.

The effect of treatment with bromelain plus papain gel on shear bond strength

Deproteinisation with 3% or 6% bromelain gel plus papain significantly increased bracket shear bond strength when phosphoric acid etching was performed, followed by primer application and attachment using Transbond XT (Group 3) and when attached using RMGIC without etching. Therefore, Groups 3 and 6 were compared with the other corresponding controls (Groups 7 and 10) and with the group in which deproteinisation was performed with sodium hypochlorite, followed by etching with polyacrylic acid and bracket attachment using RMGIC (Group 13), to evaluate the effectiveness of applying bromelain plus papain before all methods of etching and attachment. The results demonstrated that the treatment of Group 3 resulted in significantly higher shear bond strength than those of all the other treatments, except Group 10. The treatment of Group 6 resulted in the higher shear bond strength only in comparison with Group 7 (Figure 5).

The effect of treatment with bromelain plus papain gel on the Adhesive Remnant Index

Deproteinisation with 6% bromelain gel plus papain significantly increased the ARI score only when

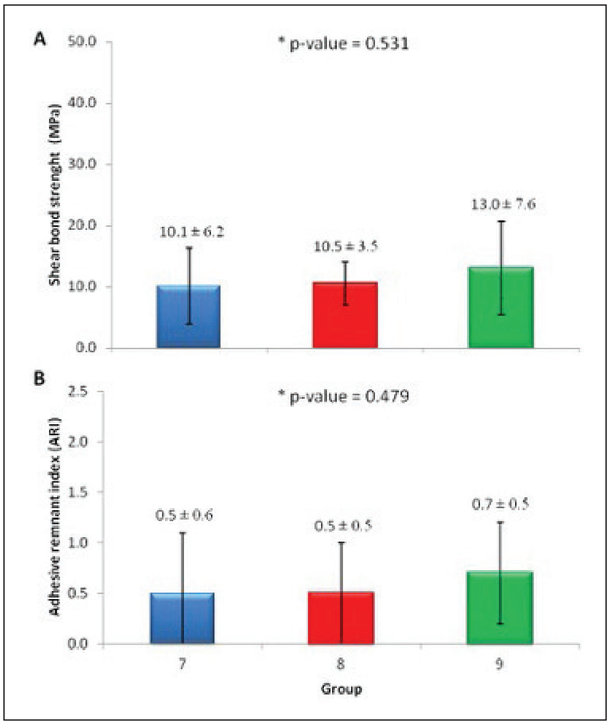

Figure 3. Comparison of shear bond strength (A) and ARI (B) between groups. Columns represent means, and error bars the standard deviations.

*Kruskal-Wallis Test; Group 7 (Control): etching with polyacrylic acid and fixation with RMGIC; Group 8: deproteinisation with 3% bromelain gel plus 10% papain plus etching with polyacrylic acid and fixation with RMGIC; Group 9: deproteinisation with 6% bromelain gel plus 10% papain plus etching with polyacrylic acid and fixation with RMGIC.

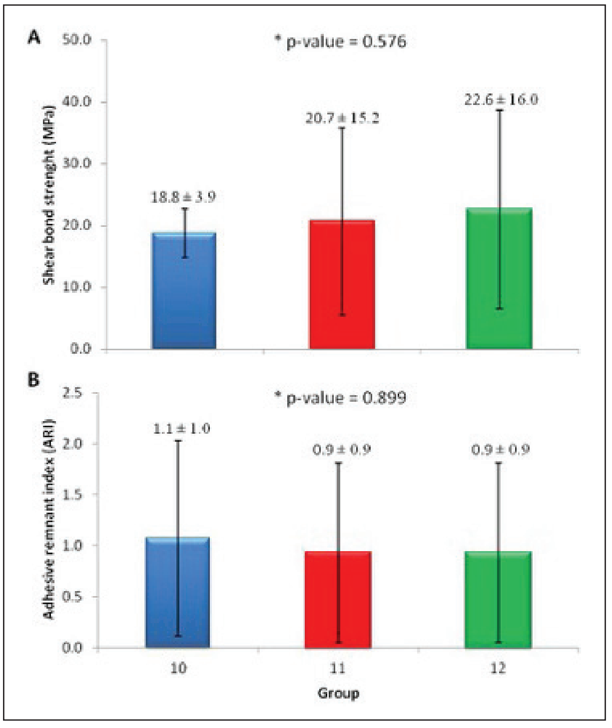

Figure 4. Comparison of shear bond strength (A) and ARI (B) between groups. Columns represent means, and error bars the standard deviations.

*Kruskal-Wallis Test; Group 10 (Control): etching with phosphoric acid and fixation with RMGIC; Group 11: deproteinisation with 3% bromelain gel plus 10% papain plus etching with phosphoric acid and fixation with RMGIC; Group 12: deproteinisation with 3% bromelain gel plus 10% papain plus etching with phosphoric acid and fixation with RMGIC.

bracket attachment was performed with RMGIC without etching. Therefore, it was decided to compare Groups 5 and 6 with the other controls (Groups 1, 7 and 10) and with the group in which deproteinisation was performed using sodium hypochlorite, followed by etching using polyacrylic acid and attachment using RMGIC (Group 13), to evaluate the overall effectiveness of bromelain plus papain. The results demonstrated that the treatments of Groups 5 and 6 did not result in a higher ARI value when compared with Groups 1, 7, 10 and 13 (Figure 6).

Discussion

The purpose of prophylaxis of the enamel surface is to eliminate organic matter that might hinder the effectiveness of enamel etching. However, some proteinaceous substances still persist on the tooth surface.[1] Therefore, it has been proposed that the additional step of enamel surface deproteinisation using proteolytic substances before acid etching would be beneficial.[1,2,27,28]

Justus et al. used 5.25% sodium hypochlorite for this purpose[27] following its efficacy in endodontic irrigation. Alternatively, enzymes present in vegetables are potent in the elimination of organic matter.[29] Pithon and collaborators[2] proposed the use of papain, an enzyme present in papaya fruit, and previously achieved statistically significant results related to the shear bond strength of orthodontic brackets and the Adhesive Remnant Index (ARI).

Bromelain is a proteolytic enzyme with similar characteristics to those of papain and is extracted from *Ananas comosus*, which belongs to the Bromeliaceae family. This enzyme may be used in association with papain for the purpose of removing protein from enamel surfaces prior to orthodontic bracket bonding. Therefore, the proposal of the present study was to verify the hypothesis that enamel surface deproteinisation with different concentrations of bromelain in association with 10% papain would increase the shear bond strength of brackets bonded with orthodontic composite and resin modified glass ionomer cement (RMGIC).

The combination of bromelain and papain in gel form was appropriate because of the likely clinical ease of management and greater application control. The use of papain at a concentration of 10% was considered because it presented the greatest potential for enamel protein removal before bracket bonding.[3] The blend of bromelain and papain is supported by their synergistic combination in dentifrices, which are effective in enamel stain removal.[17]

The results of the present study showed a significant increase in shear bond strength when a combined bromelain and papain gel was used. The increase in bond strength would likely reduce the breakage of attachments during orthodontic treatment, thereby reducing orthodontic treatment time. The ARI scores were also reduced except when enamel was etched with phosphoric acid and the brackets were attached

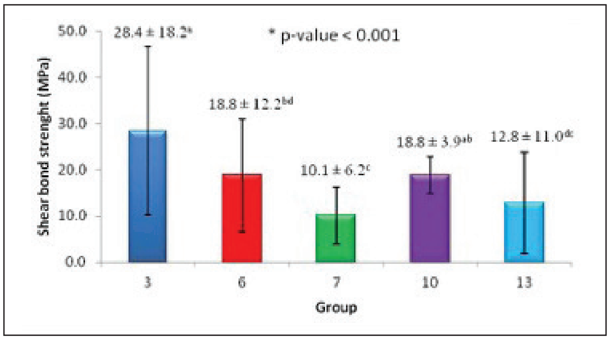

Figure 5. The effect of treatment with bromelain plus papain gel on shear bond strength in comparison with all the conditions of etching and fixation

*Kruskal-Wallis Test; a,b,c,d Values with different superscript letters indicate significant differences between the groups (Mann-Whitney Test); Group 3: deproteinisation with 6% bromelain plus 10% papain plus etching with phosphoric acid, followed by primer application and fixation with Transbond XT; Group 6: deproteinisation with 6% bromelain gel plus 10% papain and fixation with RMGIC, without etching; Group 7 (Control): etching with polyacrylic acid and fixation with RMGIC; Group 10 (Control): etching with phosphoric acid and fixation with RMGIC; Group 13: deproteinisation with sodium hypochlorite plus etching with polyacrylic acid and fixation with RMGIC.

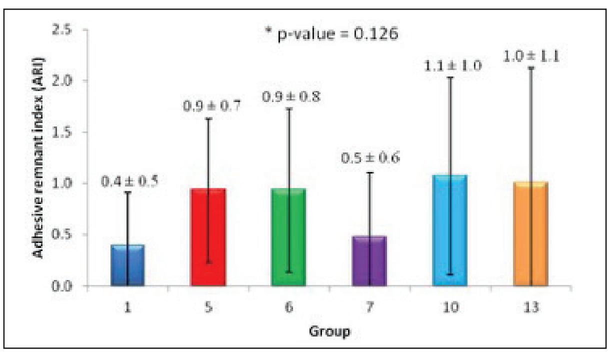

Figure 6. The effect of treatment with bromelain plus papain gel on ARI in comparison with all the conditions of etching and fixation.

*Kruskal-Wallis Test; Group 1 (Control) etching with phosphoric acid followed by primer application and fixation with Transbond XT; Group 5: deproteinisation with 3% bromelain plus 10% papain and fixation with RMGIC, without etching; Group 6: deproteinisation with 6% bromelain gel plus 10% papain and fixation with RMGIC, without etching; Group 7 (Control): etching with polyacrylic acid and fixation with RMGIC; Group 10 (Control): etching with phosphoric acid and fixation with RMGIC; Group 13: deproteinisation with sodium hypochlorite plus etching with polyacrylic acid and fixation with RMGIC.

using RMGIC. This matched the results of Pithon et al.,[2] who used papain as an enamel deproteinising agent before bonding brackets.

When brackets were attached using Transbond XT adhesive, the combination of bromelain and papain in their respective concentrations in prior deproteinisation of enamel produced superior results when compared with the use of hypochlorite.[27] When brackets were attached with RMGIC, with or without enamel etching by polyacrylic acid, the combination of the two vegetable enzymes also produced better shear bond strength when compared with pretreatment by NaOCl under the same conditions.[28]

A noteworthy finding of the present study was that deproteinisation performed with bromelain plus papain and bracket attachment using RMGIC generated higher shear bond strength compared with deproteinisation with bromelain plus papain and polyacrylic acid etching and bracket fixation with RMGIC. The results bring greater significance to the bromelain plus papain combination instead of polyacrylic acid in bonding with RMGIC.

Previous studies[2,30] have indicated that teeth etched with polyacrylic acid present significantly lower shear bond strength compared with teeth etched with 37% phosphoric acid when brackets were attached using RMGIC. This was supported by the results of the present study and also when compared with the groups in which deproteinisation was performed with papain combined with bromelain.

The groups in which an increase in the ARI index occurred when the deproteinising combination of bromelain and papain was used were those in which acid etching was performed with 37% phosphoric acid, followed by bracket attachment with RMGIC. This result requires further evaluation and explanation at a microscopic level as a decreased ARI score is desired when debonding orthodontic brackets.

The results arising from the present study bring new perspectives in the consideration of bonding orthodontic attachments to tooth enamel. However, further clinical evaluations are necessary to enable a fuller understanding of the mechanisms by which deproteinising agents act.

Conclusion

It was concluded that pretreatment enamel protein removal using bromelain combined with papain in a gel increased shear bond strength and is recommended for routine use prior to orthodontic bracket attachment.

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